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Immunohistochemical Localization of Vasoactive Intestinal Polypeptide (VIP) in Merkel Cells of Various Mammals: Evidence for a Neuromodulator Function of the Merkel Cell*

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Since met-enkephalin-like substance has been demonstrated only in Merkel cells of some rodents but not of cat, dog, pig, and humans, Merkel cells of these species were analyzed by immunohistochemistry using a variety of different antisera for the occurrence of neuropeptides different from met-enkephalin. In various locations of all species investigated Merkel cells were found to be immunoreactive exclusively to vasoactive intestinal polypeptide (VIP) but not to any of the other antisera used. Thus, in mammalian Merkel cells, neuropeptides occur that are different from met-enkephalin. It is suggested that the Merkel cell–axon complex represents a complex regulatory system involving a presumptive receptor or modulator function whereby the Merkel cell may influence the threshold of the sensory nerve ending via release of a neuropeptide (VIP- or met-enkephalin-like material).

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Abbreviations:
IR: immunoreactive
PAP: peroxidase-antiperoxidase
VIP: vasoactive intestinal polypeptide

The Merkel cell is found in the suprabasal layer of the epidermis of vertebrates. It is localized in clusters in specialized epidermal differentiations ("touch domes") preferentially at touch-sensitive areas of hairy and glabrous skin (for review see [1]). The Merkel cell is usually in close synaptic apposition [2] with a sensory intraepithelial nerve ending, constituting the Merkel cell–axon complex, a slowly adapting mechanoreceptor.

By conventional light microscopy the Merkel cell cannot be identified with certainty since it offers no staining specificity. By electron microscopy, however, the Merkel cell is easily recognized by the small membrane-bound electron-dense granules, resembling those occurring in some endocrine cells of the gastroenteropancreatic system [3] or the paraneuronal cell system [4]. Recently, it has been demonstrated by immunohis-

tochemistry that the Merkel cells of the rat [5] and some other rodents† contain a met-enkephalin-like material which is presumably located within the granules. This finding and the recent immunohistochemical demonstration of neuron-specific enolase in Merkel cells [6] supports the concept that the Merkel cell is a paraneuron and thus has a neuroreceptor or neuromodulator function in addition to other possible physiologic roles [1]. So far met-enkephalin immunoreactivity and neuron-specific enolase immunostaining are useful and, at present, the only available tools for the visualization of Merkel cells at the light microscopic level.

However, in several other mammalian species, e.g. cat, dog, pig, and human, no met-enkephalin immunoreactivity could be detected in Merkel cells.‡ Thus, the presence of a met-enkephalin-like material in Merkel cells seems not to be a general phenomenon and, therefore, is not suitable as a criterion for the identification of Merkel cells in every species. However, the definite knowledge of the chemical content of the Merkel cell granules is indispensable for a better understanding of the specific function of the Merkel cell which still is unclear. Especially in humans the determination of a specific Merkel cell neuropeptide is of crucial importance mainly with regard to the classification of the newly described group of primary malignant tumors of the skin, termed Merkel cell neoplasms [7] due to the ultrastructural resemblance of the tumor cells to Merkel cells.

The aim of the present study, therefore, was to investigate immunohistochemically whether polypeptides different from met-enkephalin are found in Merkel cells of various mammalian species including humans.

MATERIALS AND METHODS

Skin specimens from 3 dogs, 3 cats, and 4 pigs as well as skin probes of 5 men were used for this study. The dogs and cats were fixed under Nembutal anethesia by perfusion through the abdominal aorta with Bouin's fluid for 6–10 min. In pig and humans, fixation was performed by immersion of the dissected skin regions in the same fixative for 3 h. The following skin regions were dissected, dehydrated, and embedded in paraplast: the upper lips with glabrous snout and hairy skin including sinus hair follicles (cat, dog, pig), fingertip (human) and paws (cat, dog). In glabrous skin 7-μm sections were cut perpendicularly to the surface. In the sinus hair region longitudinal sections were performed through the upper sinus hair follicles bearing the Merkel cells [8,9]. The sections were processed for the peroxidase-antiperoxidase (PAP) technique [10] using the following antisera: (1) vasoactive intestinal polypeptide (VIP)-antisera R 501 (1:2000) [11], R 502 (1:2000) [12], and RRF 13/5 (1:1500) [13] which have been shown to have C-terminal specificity [11–13] and to react with identical neurons in the mammalian central nervous system [13]. (2) Met-enkephalin-antiserum 7 1/2 which has been used for the detection of met-enkephalin immunoreactive Merkel cells [5,†] (1:2000). (3) A variety of antisera against different polypeptides, i.e., β-endorphin, ACTH (34–39), α-MSH, neurotensin, somatostatin, secretin, gastrin, CCK, glucagon, and substance P (for details of the antisera and the dilutions used see [14]). To test the specificity of the obtained immunoreactions the following controls were carried out: (1) Replacement of the first antiserum by normal or preimmune serum. (2) Absorption of the VIP-antisera with 20 μg synthetic VIP per ml diluted antiserum. (3) Preincubaton of the VIP-antisera with glucagon, secretin, or glicentin. (4) Omission of single steps of the PAP-procedure.

Since all controls indicated the specifity of the obtained immunoreactions these are called VIP-immunoreactions.

RESULTS

In all species investigated Merkel cells in different localizations were VIP-immunoreactive (VIP-IR) whereas the associated sensory nerve endings gave no VIP immunoreaction. Evaluation of 5-μm consecutive sections revealed that the different VIP-antisera used reacted with identical Merkel cells. Neither in Merkel cells nor in the sensory nerve endings were any immunoreactions obtained with the other antisera applied.

In the sinus hair follicle, where Merkel cells occur in great numbers in a sheathlike arrangement in the external follicle epithelium [8,15], a high density of VIP-IR Merkel cells is present (Fig 1a). At low magnification the VIP-IR Merkel cells which exhibit variable degrees of immunostaining are recognized in the external follicle epithelium (Fig 1*a*). The VIP-IR Merkel cells are separated from each other by one or more non-IR keratinocytes. At higher magnification (Fig. 1*b,d,e*) it becomes obvious that the strongest immunoreactions derive from those cytoplasmic areas facing the intraepithelial nerve endings, which appear as clear vacuoles in parraffin sections (Fig 1*b,d,e*), or the basement membrane, i.e., from the cell poles with the highest granule density, as has been shown by electron microscopy [2,9]. However, the number of VIP-IR Merkel cells in a given longitudinal section of a sinus hair follicle is lower than the number of Merkel cells as evaluated previously by electron microscopy [9]. Probably this discrepancy is related to variable numbers of granules in different Merkel cell sections sometimes containing ony a few granules [2]. Since, as in nerve fibers or endocrine cells the VIP immunoreaction is most probably bound to the Merkel cell granules, their number sometimes may be too low to achieve visible staining in our immunoreactions.

VIP-IR Merkel cells are also encountered in the epithelial ridges of the upper sinus hair follicle (Fig 1*c*) which recently have been described by electron microscopy to contain Merkel cells [8]. Furthermore, VIP-IR Merkel cells are found in groups in the basal layer of touch domes of glabrous skin (Fig 1*d,e*) in all animals investigated. In human skin, VIP-IR Merkel cells are present only in small numbers in the basal epidermis of mechanosensitive areas of hairy and glabrous skin, corresponding to the general rare occurrence of Merkel cells in humans. Constantly VIP-IR Merkel cells are detected in the basal epidermis of the glandular ridges of the fingertip (Fig 2). This corresponds to the recent electron microscopic demonstration of Merkel cells in the glandular ridges of the finger [16].

Except for the Merkel cells, no other epidermal cell showed a positive immunoreaction either with the VIP-antisera or with the other antisera used.

DISCUSSION

The present immunohistochemical identification of VIP-like material in Merkel cells of several species demonstrates that in mammalian Merkel cells neuropeptides occur which are different from met-enkephalin. This has been postulated in another study in which met-enkephalin-IR Merkel cells in guinea pig, rat, mouse, and hamster, but not in cat, dog, pig, and humans were found.‡ In summary, our results indicate that Merkel cells from different species are either met-enkephalin-IR or VIP-IR. In no species investigated both met-enkephalin-IR and VIP-IR Merkel cells were observed. Thus, met-enkephalin-like material may be characteristic for the Merkel cells of rodents, possibly indicating a specialized line of phylogeny. In contrast, the presence of VIP-IR in Merkel cells of mammals as diverse as primates, carnivores, and suiformes may indicate a more general way of evolution. Thus, work is in progress to study the occurrence of met-enkephalin-IR and VIP-IR in Merkel cells of representatives of lower vertebrate classes in order to shed more light on these problems. Our study is the first to establish a "dual" distribution of VIP in the mammalian skin, i.e., in dermal nerve fibers [17,18] and in the Merkel cell. It should be noted that one of the antisera used (R 501) in different mammalian species, including dog and cat, reacted only with enteral nerve fibers and neurons, but not with enteroendocrine cells [19]. Thus, the C-terminal of VIP which in mammals seems to be characteristic for VIP of nerve origin [11,19] occurs in the Merkel cell but not in endocrine gut cells.

There is increasing evidence that VIP may function as a neurotransmitter, since, for example, VIP-IR occurs in perikarya and nerve fibers throughout the mammalian peripheral

‡ Hartschuh W, Weihe E, Reinecke M: Met-enkephalin-like material: the characteristic Merkel cell neuropeptide of rodents. In preparation.

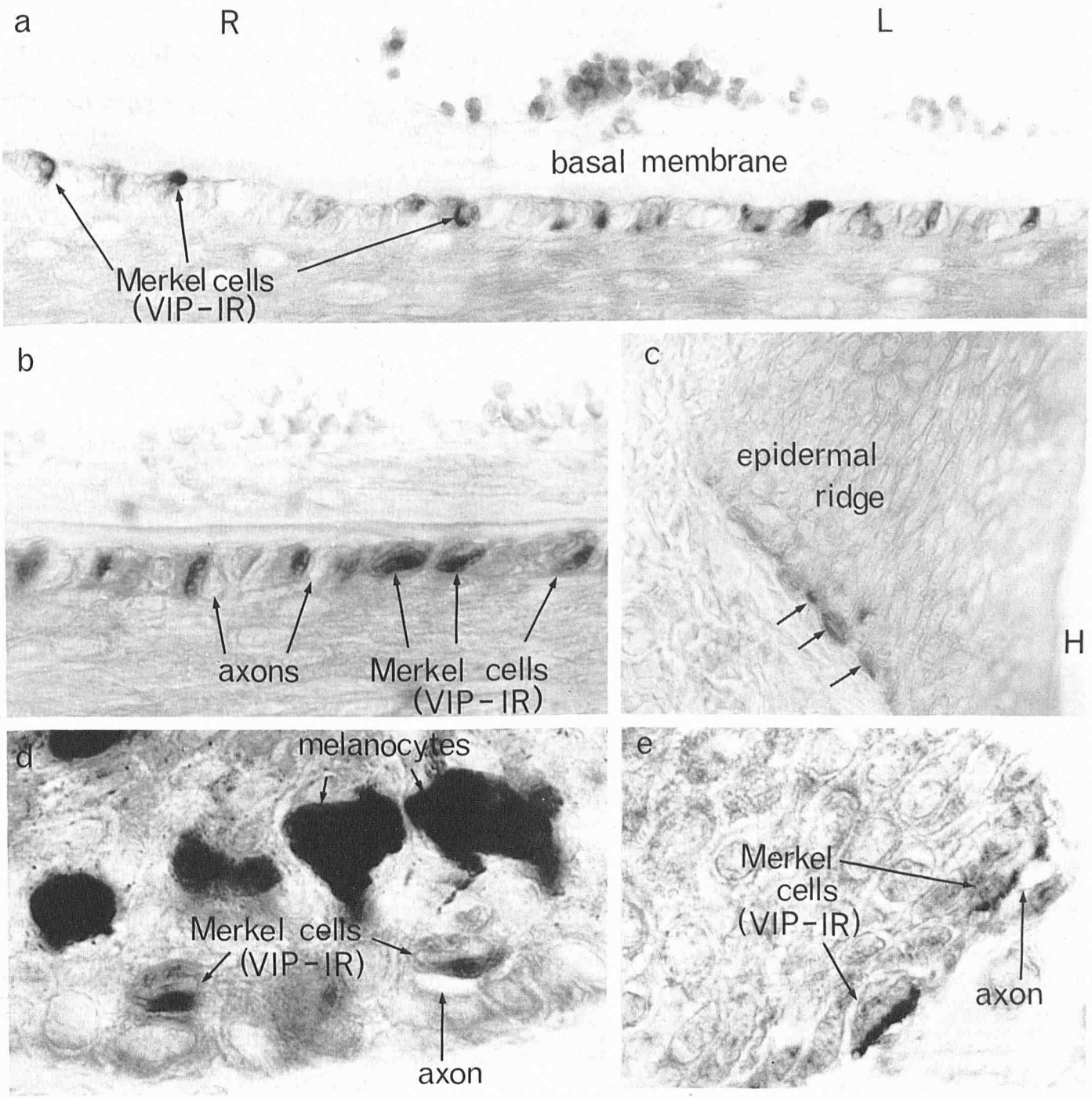

FIG 1. Immunohistochemical demonstration of Merkel cells from different species and localizations containing a VIP-like immunoreactive (VIP-IR) substance. *a*, Merkel cells in a longitudinal section of a sinus hair follicle (cat) exhibiting VIP immunoreactions of variable degrees. *R* = Ring bulge; L = lumen of the blood sinus. × 180. *b*, Higher magnification reveals that only the Merkel cells are VIP-IR, while the axons show no immunoreaction. Sinus hair follicle (dog). × 500. *c*, VIP-IR Merkel cells at the base of an epidermal ridge of the upper sinus hair follicle (dog). H = Hair. × 170. *d*, VIP-IR Merkel cells in a touch dome of the glabrous paw (cat). The strongest reaction is seen in the basal part of the cells which is known to have the highest granule density. The dark color of the melanocytes is due only to their melanin content × 880. *e*, Perpendicular section through the base of a touch dome of glabrous snout skin of pig exhibiting VIP-IR Merkel cells in the basal epidermal layer. The VIP immunoreaction is confined to the basal part of the cells. × 800.

and central nervous system [20] and VIP is released from isolated nerve endings by depolarizing stimuli [21]. Thus the demonstration of VIP-like material in Merkel cells of various mammals including humans may indicate a neuroreceptor/modulator function of the Merkel cell. However, the definite physiologic role of the neuropeptide-like materials in the Merkel cells is still unclear and is a matter of speculation since the function and significance of the Merkel cell are still enigmatic.

In recent electrophysiologic studies a receptor function of the Merkel cell is emphaticially denied [22]. The evidence is based on receptor delay measurements, the results of which seem to exclude a chemosynaptic transmission from the Merkel cell to the nerve ending. Thus it is suggested that the nerve ending represents the mechanoreceptor, and the Merkel cell merely functions as an abutment for the mechanosensitive nerve ending [22]. However, a neuromodulator function of the Merkel

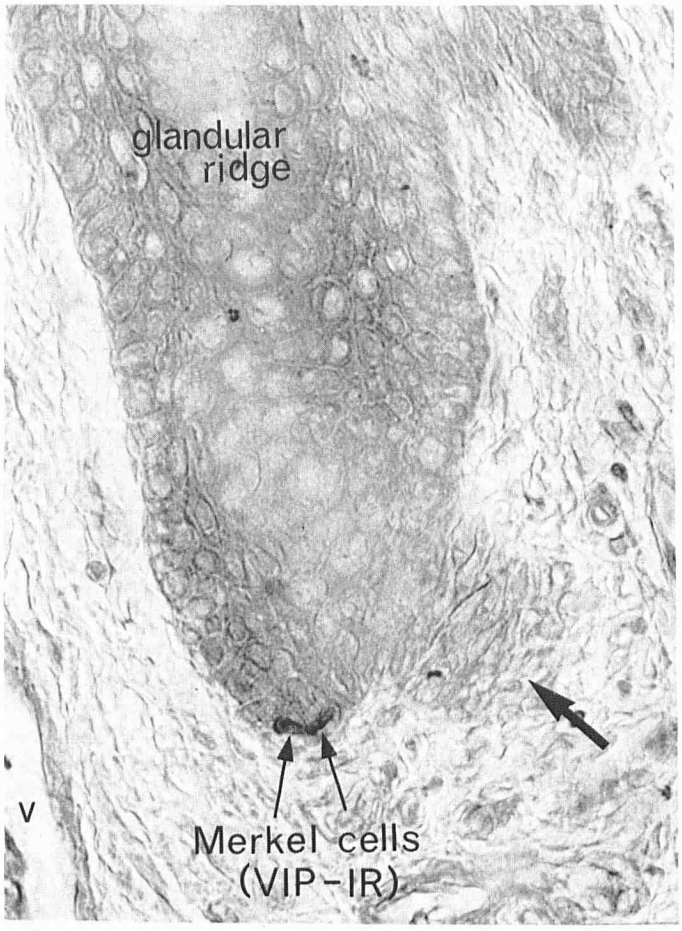

FIG 2. Ridged skin of the human fingertip showing 2 VIP-IR Merkel cells in typical localization, i.e., where a sweat gland duct is entering the glandular ridge (*thick arrow*). *v* = venuole. × 170.

cell, i.e., influencing the threshold of the sensory nerve ending via release of a neuropeptide (VIP or met-enkephalin), may not be ruled out by these experiments and should be taken into consideration with further electrophysiologic experiments.

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